The Antibacterial Activity and Corrosiveness of Certain Cleaners and Disinfectants

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ABSTRACT

Southcott, B.A., J.W. Boyd, W. Green, M. Ho, and V. Gray. 1982. The antibacterial activity and corrosiveness of certain cleaners and disinfectants. Can. Tech. Rep. Fish. Aquat. Sci. 1148.

The antibacterial activity of cleaners and disinfectants against bacterial isolates belonging to the genera Pseudomonas, Acinetobacter, Moraxella, and Aeromonas was investigated. The compounds studied included two cleaners, two iodophors, a quaternary ammonium compound, and hypochlorite. The antibacterial activity of the compounds was decreased when organic material (fish flesh extract) was incorporated into tubes of test solutions. The results from this trial indicate that separate operations of cleaning, rinsing, and disinfecting are essential to ensure effective sanitation. During accelerated corrosion tests the loss in weight of copper coupons (25 cm^2) was 82% greater during exposure to an acidic environment than an alkaline environment.

Key words: antibacterial, cleaners, disinfectants, sanitizers, corrosion.

RÉSUMÉ

Southcott, B.A., J.W. Boyd, W. Green, M. Ho, et V. Gray. 1982. L'activité antibactérienne et nature corrosive de certains nettoyeurs et désinfectants. Can. Tech. Rep. Fish. Aquat. Sci. 1148.

On a étudié l'activité antibactérienne des nettoyeurs et désinfectants contre des bactéries isolées appartenant au genre Pseudomonas, Acinetobacter, Moraxella, et Aeromonas. Les composés étudiés comprenaient deux nettoyeurs, deux composes iodés, un composé d'ammonium quaternaire et l'hypochlorite. L'activité antibactérienne des composés diminuait lorsque des matières organiques (extrait de chair de poisson) étaient introduites dans les tubes de solutions d'essai. Les resultats de ces essais indiquent que les operations independantes de lavage, rinçage et désinfection sont essentielles pour assurer un assainissement efficace. Durant des epreuves de corrosion accélérée, la perte de poids des plaques de cuivre (25 cm^2) etait 82% plus forte en exposition en milieu acide qu'en milieu alcalin.

Mot cléf: antibactérien, nettoyeurs, désinfectants, agents d'assainissement, corrosion.

OBJECTIVES OF THE INVESTIGATION

Uncontrolled proliferation of fish spoilage bacteria contributes substantially to deterioration of fish quality. Control of bacterial growth must therefore be of concern to the primary fishing industry.

Reports in the literature on the use of various cleaners and disinfectants for sanitation have been mainly concerned with the inhibition of medically important bacteria in hospital or other institutional environments. During trials on commercially available sanitation products investigators have used, as test bacteria, members of groups constituting a public health hazard; since these bacteria are not included in typical fish flora, results have not always been applicable to the fishing industry situation.

The objectives of experiments carried out by the Technology Services Branch have been to test the efficiency of cleaners and disinfectants which might be considered suitable for use in the fishing industry against bacteria isolated from fresh and spoiling fish and other marine products, and to assess the rate of corrosion of copper during exposure to acid and alkaline environments of sanitizing solutions. Copper was used since copper tubes are used in the construction of refrigerated seawater chillers.

INTRODUCTION

A. Microbiology of Fish

The goals of the fishing industry must include three interdependent points (Thatcher, 1973):

1. economic soundness of the total operation
2. organoleptic acceptability and reasonable storage life of the product
3. consumer safety.

In order to supply the consumer with high quality, safe products, the fishing industry must employ sound sanitary practices in all phases of its operations from harvesting to distribution. It has been stated (Pedraja, 1973) that a sanitation program can be no better than the attitude and

willingness of the people involved.

The processing of any food includes procedures which retard deteriorative changes resulting from microbiological and biochemical activities. In the fishing industry, problems are created by the origin and nature of the raw material, and the exigencies of fish catching and handling operations (Liston, 1970). Regardless of the initial freshness of a fish, it can be degraded by subsequent handling with unclean equipment, or inadequate storage facilities.

Following harvest and exposure to air, the death of a fish results in disorganization of the natural balance of tissue enzymes, and a series of reactions results causing a spoilage which is essentially autolysis (Shahani, 1973). Herbert et al (1971) reported that changes due solely to tissue enzyme activity in fish are considerable when expressed in chemical or biochemical terms, but are relatively unimportant organoleptically.

Thatcher (1973) stated that although degradative changes brought about by tissue enzymes are often important in the organoleptic qualities of fish, the primary and essential requirements for the production of fish of satisfactory quality and safety are:

1. to minimize bacterial contamination from exogenous sources
2. to handle and process fish in a manner which minimizes multiplication of bacteria, exogenous or endogenous.

Bacteria assume a primary role in the deterioration of fish quality as a result of their elaboration of enzymes which cause many kinds of degradation. Fish spoilage bacteria will grow slowly at or near 0 C, in contrast to pathogenic bacteria which usually require high temperatures near human body temperature (37 C) for active growth. The provision of a cool temperature is therefore the outstanding need in the preservation of the quality of freshly caught fish; low temperatures will reduce the rate of tissue enzyme activity, minimize the growth of fish spoilage bacteria, and inhibit the growth of pathogenic bacteria.

Many studies have been carried out in various areas of the world concerning the occurrence, distribution, metabolism, classification, and spoilage activities of bacteria from fresh and spoiling fish at low temperatures. Tarr (1954) reviewed early work on post mortem deterioration of fish. Shewan (1974) reported that biodeterioration of fish stored at

chill temperatures was accompanied by a marked increase, both relatively and absolutely, in numbers of bacteria of the genera Pseudomonas, Acinetobacter, and Moraxella. Shewan and his colleagues at the Torry Research Station (Aberdeen, Scotland) developed an identification scheme for asporogenous, motile, Gram-negative rods commonly isolated from marine products (Shewan et al, 1960). Lerke (Lerke et al, 1963, 1965, 1967; Adams et al, 1964) studied the bacteriology of fish muscle spoilage, and investigated the roles played by different groups of bacteria and the influence of protein on spoilage patterns. Levin (Levin, 1968; Chai et al, 1968) investigated the role of certain pseudomonads in spoilage of fish. Miller et al (1973) identified volatile compounds produced in fish muscle by certain species of Pseudomonas. Lee and Pfeifer (1974) studied the influence of temperature on the type of bacteria isolated from seafoods.

The results of these investigations emphasize the ubiquitous nature of fish spoilage bacteria, their wide temperature tolerance, and the diversity of their metabolic activities leading to production of compounds involved in the deterioration of fresh fish.

The Inspection Division of the Field Services Branch, Department of Fisheries and Oceans Canada, monitors handling and processing procedures on board vessels and in plants, and microbiology of finished products, both domestic and imported, in order to protect the consumer from products posing a health hazard. Inspectors check vessels and plants for compliance with construction and equipment standards, and identify areas where contamination of the product could take place during handling and processing. Samples of partially processed and finished fishery products are examined by the Inspection Laboratory for numbers and types of microorganisms present - total counts and, for example, tests for presence of coliforms and members of the genera Salmonella, Shigella, Vibrio, Staphylococcus, and Streptococcus may be carried out. The continuing inspection of fishery products does not, however, give separate consideration to the fish spoilage psychrotrophic (able to grow at refrigerator temperatures) bacteria which, from the time of fish harvest, can exert a deleterious effect on the organoleptic acceptability of the final product.

In production of high quality fish, it is mandatory that proper sanitation procedures be followed so that there will be no carry over of

bacteria from one load of fish to a subsequent fresh load. The fact that fish spoilage bacteria are psychrotrophic means that ice or refrigerated seawater will not destroy them; it is therefore necessary to reduce the numbers of fish spoilage bacteria by a satisfactory sanitation program. Tarr (1947) studied chemical disinfection and corrosion prevention in the fishing industry. The problem of cleaning fish boxes has been addressed by Spencer (1959, 1960, 1961), Kochanowski (1964), and Bhadra and Bose (1975). Problems of sanitation in the fishing industry have been reviewed by Liston (1970), Lane (1970), Tatterson and Windsor (1970), and Lee (1973).

B. Sanitation

Various terms are used throughout the literature and in industry to designate substances and procedures used in the sanitation of food processing equipment. Definitions of certain terms follow:

Sanitizer

To sanitize usually means to reduce the microbial population to an acceptable level as judged by public health standards. The term implies a process consisting of two operations - cleaning and disinfecting. "Sanitizer" commonly refers to a compound with these two activities, but in some advertising material the term may mean only disinfection. A successful sanitation program must embody procedures leading to:

1. clean and microbiologically satisfactory conditions
2. freedom from toxic residues
3. absence of problems related to economics, storage of concentrated solutions, corrosion, foaming, and effluent disposal.

Cleaner

To clean means to remove visible soils, fish residues, and foreign matter from surfaces. Cleaners may be applied by soaking, brushing, spraying, or circulation. Cold water, hot water, and steam, under pressure when necessary, may be used for cleaning; acids and alkalis may be needed for special purposes. Soaps, made from fats and lye, are not generally used

because of the problem of redeposition of solids. Detergents are often employed to assist in the cleaning process.

Detergent

A detergent is a chemical agent (other than soap) which helps the cleaning action of water. Detergents contain varying components depending on their use, but generally consist of a combination of the following:

1. cleaning agent - comprises alkaline salts or synthetic compounds
2. surfactant - provides wetting ability which increases the action of other ingredients and allows water-insoluble substances to be lifted by the cleaner into water in a suspended or emulsified form
3. chelating sequestering agent - binds hard water factors in solution so that they will not form insoluble deposits with proteins present.

A correctly selected detergent will be seen to be effective under specific conditions of use, will be safe and non-corrosive, and will not affect the odour or flavour of the product.

Disinfectant

A disinfectant is a chemical agent which is capable of destroying pathogenic and other microorganisms, but not necessarily bacterial spores; the term usually applies to a substance used on inanimate objects. Bactericides, fungicides, and germicides are all disinfectants. A disinfectant under conditions unfavourable to its action may be bacteriostatic rather than bactericidal, that is, bacteria may be stopped from reproducing but not killed. Commonly available disinfectants include chlorine-releasing agents (inorganic or organic), iodophors, and quaternary ammonium compounds.

Sterilant

The term sterilant is sometimes used interchangeably with disinfectant. In a strict sense, the term sterile means free from all living matter, including spores, and should therefore be restricted to use in reference to medical and hospital procedures.

Antiseptic

An antiseptic is a disinfectant which can be used on living human tissue. The term may refer to substances which are bactericidal or bacteriostatic.

The types of soil to be dealt with in the fishing industry may be placed in three groups: mineral deposits, organic soil, and living microorganisms.

1. Mineral deposits - comprise insoluble salts ("stone") formed by the reaction of ions present in hard water or cleaning compounds with fish protein debris
2. Organic soil - includes the lipids, proteins, and carbohydrates present in fish debris, and deposits of dead microorganisms
3. Living microorganisms
   (a) Endogenous bacteria - are those naturally present in the slime, gills and gut of fresh fish, belonging in general to the genera Pseudomonas, Acinetobacter, Moraxella, Vibrio, and Aeromonas. The potential fish spoilage bacteria are not necessarily actively proteolytic; as a result of their metabolic activity, characteristic spoilage compounds are produced which accumulate and seriously affect the organoleptic qualities of the fish. Great economic loss may result from proliferation of these bacteria. Bacteria endogenous to fish are seldom pathogenic and do not create health hazards; however, the food-poisoning species Clostridium botulinum and Vibrio parahaemolyticus may occur on freshly caught fish in some areas of the world (Southcott et al, 1976).
   (b) Exogenous bacteria - are those which do not occur naturally on fresh fish, but rather are present as a result of contamination from polluted seawater or from human or animal sources during handling and processing of fish. This group includes coliforms, staphylococci, streptococci, and salmonellae.

Sanitation programs in the primary fishing industry must include measures to deal with all types of soil. Occasional treatment with acid cleaner may be necessary to remove accumulated mineral deposits. Cleaners in routine use should be selected for their ability to remove organic soil and prevent redeposition. Living bacteria may be attached to fish or to organic soil; many bacteria may be detached physically when organic soil is removed by cleaning, but even a few remaining bacteria may subsequently grow sufficiently to contaminate the next batch of fresh fish. Davis (1962) has stated that efficient cleaning removes 99% of the bacteria on a dirty object, while Stinson and Forwalter (1978) reported that effective cleaning of equipment is 90% of the total sanitizing job.

Disinfection in the primary fishing industry must have as its objective the destruction, or at least inhibition, of potential fish spoilage bacteria, and the maintenance of equipment in a disinfected state to prevent contamination of fresh fish. The destruction of bacterial spores is not always achieved by routine disinfection procedures; for example, spores of C. botulinum a hazardous food-poisoning bacterium, must be prevented from growth and toxin production by judicious application of temperature control in products at risk (Southcott and Razzell, 1973).

C. Corrosion

The corrosion of metals may be broadly defined as any chemical or electrochemical reaction between them and their environment which results in their deterioration and destruction (Rogers, 1960). Work by metallurgists on the fundamentals of corrosion has shown that the essential phenomena are the same for all metals and alloys differing in degree but not in kind; in aqueous corrosion the driving force of the corrosion reaction between a metal and its environment is electrochemical. The rate of reaction is controlled by many factors such as temperature, salinity, oxygen, pH of the electrolyte, and the type of metal. Loss in weight, color change, pitting, and blistering are some of the changes which may occur to a metal such as copper when in contact with a corrodent such as a liquid, or a gas, or a combination of the two. In the fishing industry copper tubing is used in the construction of heat exchange units which are used to chill sea water in refrigerated

seawater (RSW) systems (Roach et al, 1967). Following the unloading of fish from RSW tanks it is recommended that the tanks and units be cleaned and disinfected with suitable compounds to eliminate the possibility of contaminating the next load of fish with fish debris and spoilage bacteria (Gibbard and Roach, 1976). It was also suggested that such compounds should be closely investigated to assess their corrosiveness to metals (e.g. copper) in RSW systems. The compounds should be effective for their specific application, but should not present an environment in which the rate of corrosion of copper is increased beyond acceptable limits.

METHODS

Bacteria

Bacteria were isolated from such sources as cod, salmon, sole, prawns, and herring roe on streaked or poured plates of nutrient agar, nutrient agar plus 3.0% NaCl, or Eugonagar (BBL) at 4 C, 14 C, or 25 C. Isolates were purified by streaking onto Eugonagar plates; stocks were maintained on Eugonagar slants with monthly transfers.

Classification tests were carried out, using those procedures which would allow assignment of isolates to genera according to the scheme developed by Shewan et al (1960); additional tests were performed when necessary (Lerke et al, 1965; Stanier et al, 1966; Baumann et al, 1968 a,b). Isolates selected for use in the testing of cleaners and disinfectants belonged, based on their principal characteristics, to the genera Pseudomonas, Acinetobacter, Moraxella, and Aeromonas. Isolates from fish do not always correspond completely to literature descriptions of the genera, which have usually been based on isolates from sources other than marine. Standard reference stock cultures were also used: Pseudomonas fluorescens and P. fragi from the American Type Culture Collection, and P. putrefaciens from the department of Microbiology, University of British Columbia.

Bacteria to be employed for tests of cleaners and disinfectants were taken from stock and transferred serially several times in broth, if possible at the temperature to be used in the test.

Fish Juice

Fish juice preparations were used during the testing of cleaners and disinfectants for antibacterial activity in order to simulate the presence of fish debris and other organic material.

Cod or salmon fillets were skinned, then comminuted in a domestic food grinder. Batches of 100 g were placed in individual plastic bags and frozen; bags of fish were removed and thawed as necessary for preparation of fish juice.

Comminuted fish and water, in the desired proportion (usually 1:1), were brought to a boil, filtered through a double layer of cheesecloth and then through Whatman No. 4 filter paper. The juice was autoclaved in 25 ml lots in 50 ml screw-capped tubes at 15 lb pressure for 15 min, then stored at 4 C until used.

Cleaner Tests

The actual "cleaning" ability of commerically available cleaners was not tested since cleaners formulated by numerous manufacturers to cope with various specific industrial requirements would be expected to be efficient physical cleaners, that is, would remove visible soils from dirty surfaces. Cleaners do not necessarily have any antibacterial activity, but any such action would be a positive factor in the total sanitation of equipment.

The antibacterial activity of certain cleaning compounds was determined by exposure of test bacteria to a range of concentrations of the cleaner (usually 0-30%) prepared by dilution of the cleaner with tap water or with fish juice. Routinely, one drop of an overnight broth culture was added to tubes containing 5 ml of diluted cleaner, the test bacterium was exposed for 1 or 2 hours, then 10 drop transfers were made to recovery tubes containing 5 ml of Eugonbroth; the broth tubes were incubated to determine the presence of viable bacteria.

Long exposure periods were used in order to allow any antibacterial activity to manifest itself. The dilution of the transfer in the recovery broth simulated the rinsing off of the cleaner after the exposure period.

Disinfectant Tests

The antibacterial activity of certain disinfectants was determined by an adaptation of the Capacity Use Dilution Test (Kelsey and Sykes, 1969; Bergan and Lystad, 1971). This method determines the lethal activity of a given concentration of a disinfectant upon successive additions of bacteria and organic material. In a 35 min period, three successive 1 ml additions of bacteria suspended in fish juice (or in physiological saline for control) are made to 3 ml quantities of four different concentrations of a disinfectant; transfers are made, at 8 min intervals after each addition, to recovery medium containing a disinfectant inactivator to prevent carry-over of antibacterial activity. Required manipulations are outlined in Table 1.

Growth observed in recovery tubes at 24 h or less is significant in that it indicates presence of active bacteria in the test mixture of disinfectant and bacteria subsequent to the exposure interval; such bacteria could start to grow immediately if a piece of presumably disinfected equipment was again soiled with fish. Growth observed in recovery tubes after a longer incubation (48 h), or after removal to a warmer temperature following the initial incubation, would indicate the presence of damaged bacteria which could develop under certain conditions of storage, temperature, and soiling of the disinfected equipment.

As successive additions of bacteria-fish suspension are made to the reaction tubes, the concentration of disinfectant falls and the concentration of bacteria and organic material increases. Bergan and Lystad (1971) considered that the lowest disinfectant concentration safe for use was that which permitted two incremental additions before a positive culture appeared. In other words, an acceptable concentration in reaction tubes A to D (Table 1) would allow no growth in subcultures i and ii. These authors concluded that the concentrations of disinfectant recommended by most of the manufacturers for the products they tested were only about one-half of those presumed safe from the Capacity Test. The Capacity Test simulates realistically practical conditions, with maximum loads of bacteria and organic soil.

MacKinnon (1974), in a study of inactivators used in the recovery tubes for disinfectant evaluation, concluded that many traditionally used inactivators are inhibitory to damaged organisms; he recommended a non-ionic

surfactant (Tween 80) for testing iodophors. The recovery medium used in investigations at this laboratory was Letheen Broth (Difco), composed of lecithin and Tween 80, in which all test bacteria were able to grow luxuriantly.

Corrosion

Various methods are used in determining the corrosion rate of a metal, one reliable way is to expose the metal to the environment under service conditions and to assess the corrosion by means available in that particular case; in nearly every case this procedure is slow and costly which may be overcome by simulating conditions and accelerating the rate (Thompson, 1971). A few grams of metal immersed in a solution in a laboratory beaker is a method sometimes used by metallurgists to predict the corrosion rate which might occur in some large and complex equipment.

In this study copper test coupons (25 cm^2) were used in accelerated storage tests to assess their rate of corrosion in solutions of sanitizers. In preparing coupons the edges were wet polished with 180 grade carbide paper to remove burrs and roughness, methanol and HCl-18% were used to remove grease and surface film respectively, followed by a light scrub with a brush under distilled water, and then dried in an oven to a constant weight to the nearest 0.1 mg. For each test a minimum of 2 coupons were placed in a special support to prevent contact between coupons, and then immersed in respective temperature equilibrated solutions contained in 3 L capacity pyrex jars with covers. The solutions which were tested are listed in the following table.

	Concentration-%	pH
Water		6.4
Iodophor Y (-H_3PO_4)	0.5	4.4
Iodophor Z (+H_3PO_4)	0.6	3.8
Cleaner C	1.25	11.7
Cleaner D	0.35	11.0

Cleaners C and D with pH values in the range of 11 were used in this part of the trial rather than cleaners A and B which have lower pH values in the range of 8.

In an attempt to accelerate the rate of corrosion, tests were carried out at 40 C, and the solutions were agitated by means of a magnetic stirrer operating at approximately 250 rpm. Following 1 hr immersion periods coupons were removed from solutions, rinsed with water, treated with HCl to remove corrosion products, rinsed with water, dried, and then weighed to ascertain the loss in weight. Coupons were stored in a dry environment after each weighing until the next immersion period; 12 immersion-emersion cycles were carried out in this study.

RESULTS AND DISCUSSION

Cleaners

In the cleaning process, surfaces should be scrubbed or pressure-hosed, following application of the cleaning compound, in order to physically remove all organic material. Any fish debris remaining will harbour bacteria which may not have been damaged by the cleaner; the organic material will render these bacteria more resistant to a subsequent disinfection procedure.

The pH values of cleaners are usually in the alkaline range, and fish debris (simulated in these experiments by the fish juice used to prepare cleaner dilutions) will lower the pH values sufficiently so that active bacterial growth will take place. A water solution of a cleaner will usually be of a high pH until quite dilute when compared to the fish juice solution. Thus, the presence of fish debris, as well as protecting bacteria from the physical and chemical action of a cleaner, may provide a suitable pH for bacterial growth. Examples of the effect of fish juice on the pH values of typical commercially available cleaners (A and B) are given in Figures 1 and 2.

The length of the exposure interval of bacteria to cleaner has some effect upon the extent of antibacterial action. For example, when a Pseudomonas type II isolate was exposed to cleaner A for 1, 2, or 24 h in water or fish juice, growth was observed in recovery tubes as recorded in

Table 2. In industrial practice, exposure of bacteria to cleaners would probably be of much shorter duration than in laboratory tests. However, even with long exposure, cleaner A did not have antibacterial activity at levels below 18%. The manufacturer's recommended usage levels for cleaner A were 3.0 to 12.5% for different operations; these levels might satisfactorily clean physically, but would have no extensive antibacterial activity.

In the case of cleaner B, there was no antibacterial activity against a Pseudomonas type II isolate after 2 h exposure (Table 3); considerable activity was evident against an Acinetobacter-Moraxella isolate when the cleaner was dissolved in water, but not when fish juice was the solvent (Table 4).

The temperature during a cleaner test and the temperature of the recovery tubes can have an extensive influence on the outcome. If recovery tubes showing no growth at low temperatures (4 C or 14 C) are subsequently incubated for a further 24 h at 25 C, many of the negative tubes will show growth. This indicates extensive bacteriostatic activity by the cleaner in question. The degree of bacterial contamination also affects the antibacterial activity of a given concentration of a cleaner. Table 5 illustrates the effects of temperature and of the number of bacteria present upon the activity of cleaner A against Pseudomonas fluorescens (Pseudomonas type I). A bacteriostatic effect is evident. The presence of fish juice during the exposure interval has provided protection for the bacterial cells, thus ensuring the existence of more undamaged cells in the recovery tubes. When a similar test was conducted at 4 C, growth in recovery tubes at 4 C occurred only where transfers had been from exposure tubes containing fish juice and cleaner levels of 6.0% or less.

The effect of the amount of fish debris present in exposure tubes is shown in Table 6, where fish : water proportions of 1:1 and 1:2 are compared. On the basis of cleaner test results using isolates of Pseudomonas (types I, II, and III/IV), Acinetobacter-Moraxella, and Aeromonas, it is evident that these genera, common on spoiling fish, are subject to bacteriostatic effects depending upon circumstances of temperature, exposure time, and presence of organic material. Members of the Acinetobacter- Moraxella group appear less resistant than the other genera tested.

The results of these cleaner tests emphasize that a disinfecting procedure must follow the cleaning operation.

It is apparent that a cleaner selected for industrial use must be employed at a level sufficient to remove all soil. It has been shown that although bacteria may be damaged by cleaners, they are not necessarily killed, and that damaged cells may revive if given time and favorable growth conditions.

Thorough rinsing is mandatory after cleaning but before disinfection to ensure that fish debris, killed bacteria, and viable bacteria have been removed to the greatest extent possible. Fish debris remaining on equipment may retain viable bacteria, protect them from subsequent disinfectant action, provide a medium for bacterial growth, and exert an antagonistic effect toward disinfectants.

Disinfectants

In order to achieve the maximum antibacterial activity of disinfectants, the objects to which they are applied must be clean. As demonstrated in tests described in the preceding section, cleaners may have some antibacterial activity, but cannot be relied upon to destroy all bacteria which might cause problems in fishery products. A disinfectant can render a surface or a piece of equipment free of bacteria only if the conditions of its use are reasonable; the factors affecting the activity of disinfectants are those which also affect cleaners - presence of organic material, pH, length of exposure, and temperature.

Quaternary ammonium compounds (QAC's) are active antibacterials, but are relatively expensive when compared to some other products available for the purpose of disinfection. QAC's must not be used with incompatable ionic detergents, and are therefore often manufactured in formulations combining detergent and disinfectant; with such products, thorough cleaning is not done before the disinfectant is applied, and organic material will affect the antibacterial activity. QAC's must be used at somewhat higher levels against Gram negative bacteria (such as fish spoilage genera) than against Gram

positive bacteria (such as Staphylococcus and Streptococcus). QAC's have poor rinsability, but this may in some cases, especially medical situations, be desirable where bacteriostatic levels of QAC's remain on sanitized objects.

When tests using a commerically available QAC were done at this laboratory, levels of the active ingredient (alkyldimethylbenzylammonium chloride) from .0025% (25 ppm) to 0.16% (1600 ppm) were used. Isolates of Pseudomonas (types I, II, III/IV) could be controlled by between 600 and 1200 ppm, depending upon the amount of organic material present. The results presented in Table 7 were obtained using the Capacity Use Dilution method, described under Methods, and a Pseudomonas type I isolate; two additions of a bacteria-fish suspension could be made without the appearance of growth in recovery tubes. The QAC showed an antibacterial effect at 1000 ppm when bacteria were suspended in saline; the presence of fish juice reduced its activity. In contrast, results from tests (not tabulated) on Acinetobacter-Moraxella isolates showed that they were very sensitive to the QAC used, and under the same conditions were controlled by 25-200 ppm depending on environmental factors.

Various chlorine-releasing substances have been widely used as disinfectants in the food industry because of their relatively low cost and their rapid effectiveness over a wide spectrum of microorganisms. Dussault (1957) outlined methods for the use of chlorine in fish plants; Spencer (1960) reported on the effectiveness of chlorine in cleaning fish boxes. Chlorine is most effective, but also least stable, at low pH's. Depending upon the compound and concentration used, problems may arise with corrosion, product off-flavours, and worker safety; correct usage will eliminate these difficulties.

Recent reports have indicated that if chlorine is used to treat water with high organic loads, the chlorinated organic compounds formed are toxic to biological systems and resistant to degradation. There is increasing concern over health hazards which may result from chlorine contact with foods (Cunningham and Lawrence, 1977). The formation of chlorinated organic compounds would arise more as a result of wastewater treatment than from chlorination of previously cleaned equipment in a fishery operation

(Dugan, 1978); however, some curtailment of the use of chlorine-releasing products may be anticipated.

A level of 500 ppm hypochlorite appeared to kill Pseudomonas fluorescens when tested at 25 C, but longer incubation showed that the action had been bacteriostatic, not bactericidal (Table 8). The results were the same when the tests were performed at 14 C.

Iodophors are complexes of iodine with wetting agents, the latter acting as carriers and solubilizers; iodophors are used because iodine itself, although a very effective disinfectant, is toxic and corrosive. Iodophors are active against a wide spectrum of bacteria, have detergent properties, do not form toxic compounds with organic materials, but may be relatively expensive depending upon the formulation used. Low pH values enhance the antibacterial properties of iodophors, but do not affect their stability, as is the case with hypochlorites. This characteristic allows iodophors to be combined with acid, usually phosphoric acid, which increases bactericidal activity. However, when iodophors are formulated with phosphoric acid, the problem of corrosion of equipment may be a serious disadvantage. Iodophor treatment was found more effective than chlorine treatment at low temperatures (Gray and Hsu, 1979).

Tests were carried out at this laboratory with commercially available iodophors formulated with and without phosphoric acid. Table 9 indicates pH changes resulting from dilution of the iodophors, as in the Capacity Use Dilution Test, by addition of 1 ml increments of saline or fish juice.

When iodophor Y (no phosphoric acid) was tested for antibacterial activity, concentrations of 40-240 ppm TI (0.25-1.5% iodophor) were employed in presence of fish juice and saline. Isolates were found to be resistant to considerably higher levels of TI in the presence of fish juice; results were affected by the amount of bacterial contamination and temperature during exposure and recovery. Many isolates which could be controlled by 40 ppm TI when suspended in saline required 240 ppm TI for control when suspended in fish juice; this level is higher than that recommended by the manufacturer (15-80 ppm TI; 0.1-0.5% iodophor). Table 10 presents typical results; using three levels of contamination by a Pseudomonas type III/IV with and without

fish juice, it is apparent that the organic matter has more effect on the results than does the degree of bacterial contamination. Extensive bacteriostatic activity by iodophor Y is evident in the presence of fish juice; satisfactory bactericidal action occurs only when the bacteria are suspended in saline. That these activities are due to the antibacterial effect of the iodophor itself and are not only the result of pH changes is evident from the data presented in Tables 9 and 10; of recovery tubes inoculated from treatment tubes of the same pH, growth occurred only in those with lower ppm TI.

Iodophor Z, commercially formulated with phosphoric acid, is recommended by the manufacturer for use at a level of 0.6% iodophor (96 ppm TI); the pH's of iodophor Z in saline (Table 9) are lower than those of iodophor Y, but in fish juice there is less difference in the pH's of the two products. Table 11 presents results of tests with the two iodophors using a Pseudomonas type I isolate.

Corrosion

The corrosion rate of metals is usually a linear function in relation to time, and environmental conditions such as temperature, pH, and concentration of chemical compounds. The results from this study are summarized in Fig. 3; they are plotted at 2 hr intervals rather than hourly. In relation to time the corrosion of copper under accelerated conditions in solutions of cleaners and disinfectants was found to be linear.

In expressing the rate of corrosion of metals metallurgists use various units to indicate a loss in mass - mpy (mils/year), mdd ($mg/dm^2/day$), and SI units ($\mu g/m^2/sec$). Three rate classes have been established by investigators to indicate the extent of corrosion: A - less than 5 mpy, B - 5 to 50 mpy, and C - over 50 mpy which is generally considered unsuitable. In converting the weight loss of coupons from Fig. 3 into mpy, and assigning each rate to a class it was found after 12 immersion-emersion cycles that none of the sanitizers was considered to be a class C corrodent; the rates and classes are presented below.

Solution	Corrosion Rate mpy	Class
Water	4.05	A
Iodophor Y ($-H_3PO_4$)	29.35	B
Iodophor Z ($+H_3PO_4$)	39.40	B
Cleaner C	4.70	A
Cleaner D	7.40	B

The combined average rate of corrosion of copper coupons during immersion in alkaline cleaners C and D was 82% less than that of coupons which were immersed in acidic iodophor sanitizers. The rate of corrosion of copper in the alkaline cleaner C was similar to that of samples in water.

The results obtained from laboratory scale corrosion trials should be thoroughly assessed prior to the use of any metal or compound in a service environment. As reported by Thompson (1971), if a laboratory test indicates a corrosion rate of 5 mpy, it is unwise to conclude that a piece of metal with a thickness of 1.27 mm (0.05 in.) will last 10 years. However, a high corrosion rate from a laboratory scale trial would indicate that either the metal or the environment is not suitable for application to service conditions.

CONCLUSIONS

Several types of Gram-negative, asporogenous bacteria were isolated from various species of fish, and were found to belong to the genera reportedly associated with problems of fish spoilage. These isolates were able to grow at low temperatures (psychrotrophic), even though they did not show optimum growth at such temperatures, as do psychrophiles.

Experiments with 2 iodophors, 2 alkaline cleaners, a quaternary ammonium compound, and hypochlorite demonstrated that none of the isolates was completely resistant to the antibacterial action of the products tested. The antibacterial activity of cleaners and disinfectants was affected principally by the amount of interfering organic material; other factors influencing the susceptibility of bacteria were the nature of the product used, the temperature of reaction and recovery, and the pH of the reaction solutions.

It has been shown that, in the planning of a sanitation programme for any fishery operation, there must be an appreciation of the factors affecting the efficiency of the products used, and especially of the interfering effect of fish debris. The importance of the separate operations of cleaning, rinsing, and disinfecting has been emphasized. If the equipment to be disinfected has not been thoroughly cleaned and rinsed, concentrations of the disinfectant used must be higher than those recommended by the manufacturer.

The rate of corrosion of copper coupons was faster and greater when specimens were immersed in solutions of acidic compounds than in solutions of alkaline compounds. In industrial applications the corrosive properties of a sanitizer to metals should be thoroughly evaluated prior to application.

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TABLE 1

Time schedule for testing 4 disinfectant dilutions simultaneously

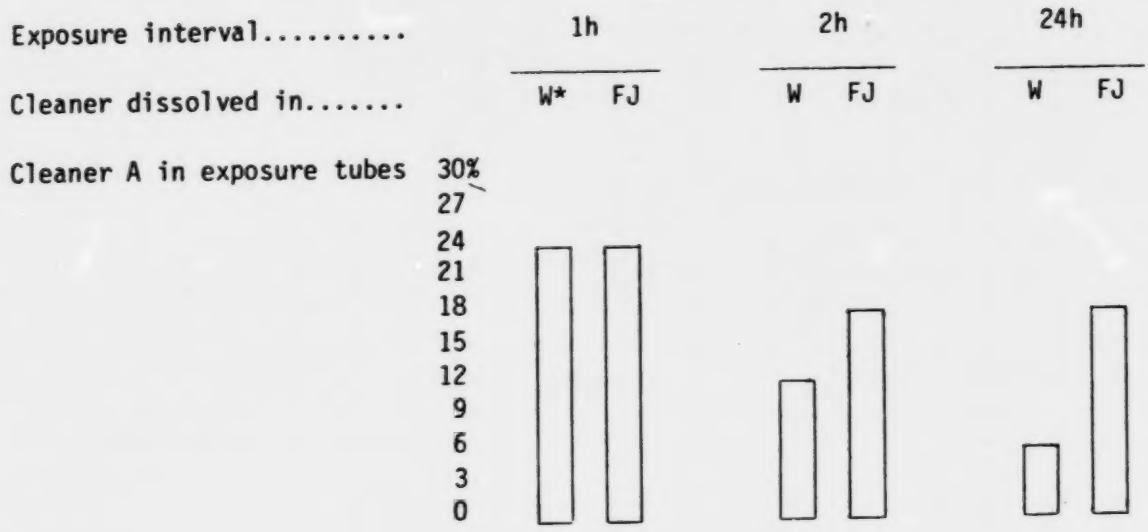
Recovery tube	Disinfectant concentration							
	A		B		C		D	
	Interval* (min)	Action	Interval (min)	Action	Interval (min)	Action	Interval (min)	Action
	0	Add 1 ml bacteria-fish suspension	1	Add 1 ml bacteria-fish suspension	5	Add 1 ml bacteria-fish suspension	6	Add 1 ml bacteria-fish suspension
i	8	Subculture	9	Subculture	13	Subculture	14	Subculture
	10	Add 1 ml bacteria-fish suspension	11	Add 1 ml bacteria-fish suspension	15	Add 1 ml bacteria-fish suspension	16	Add 1 ml bacteria-fish suspension
ii	18	Subculture	19	Subculture	23	Subculture	24	Subculture
	20	Add 1 ml bacteria-fish suspension	21	Add 1 ml bacteria-fish suspension	25	Add 1 ml bacteria-fish suspension	26	Add 1 ml bacteria-fish suspension
iii	28	Subculture	29	Subculture	33	Subculture	34	Subculture

* From start of test. Adapted from Bergan and Lystad (1971)

TABLE 2

ANTIBACTERIAL ACTIVITY OF CLEANER A

Pseudomonas type II 25C



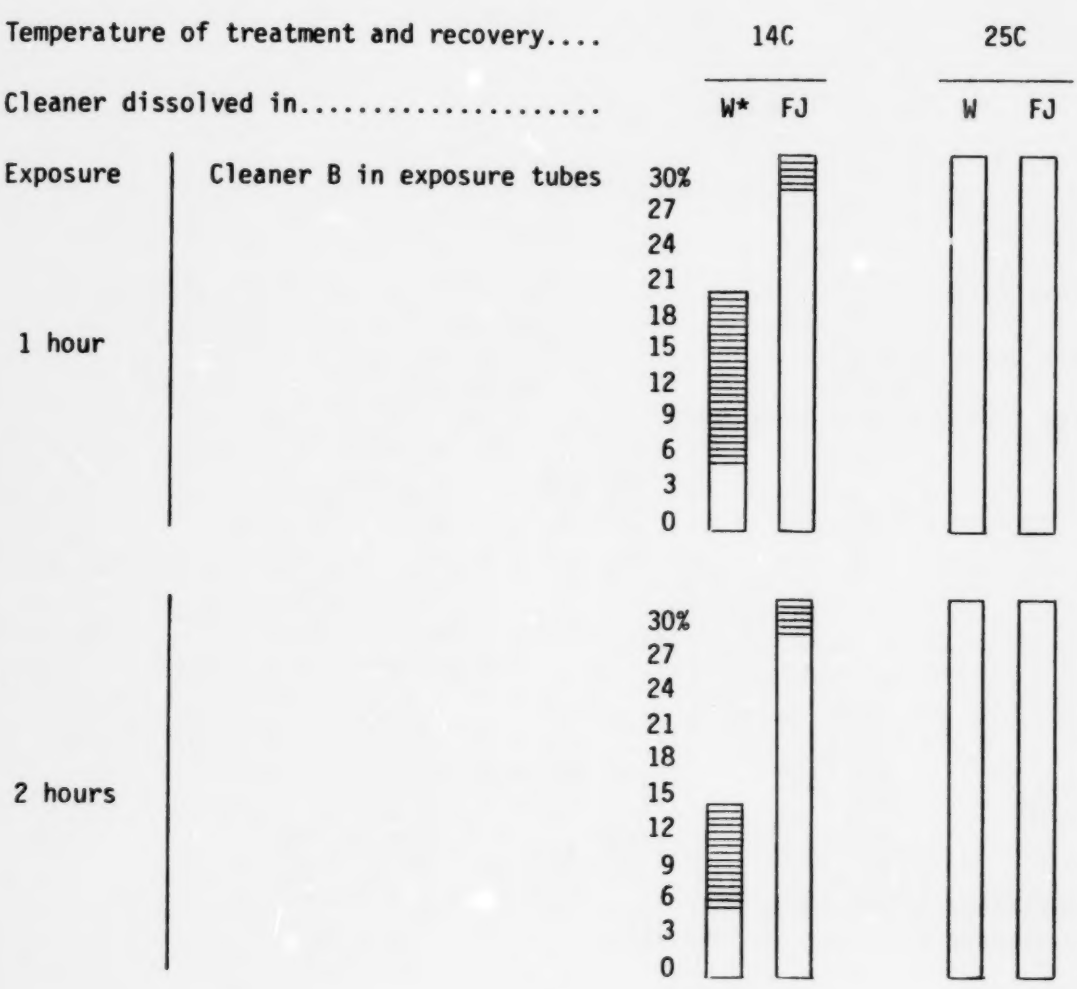
* W = cleaner dissolved in water
FJ = cleaner dissolved in fish juice

☐ = growth in recovery tubes

TABLE 3

ANTIBACTERIAL ACTIVITY OF CLEANER B

Pseudomonas type II 14C and 25C



* W = cleaner dissolved in water
FJ = cleaner dissolved in fish juice

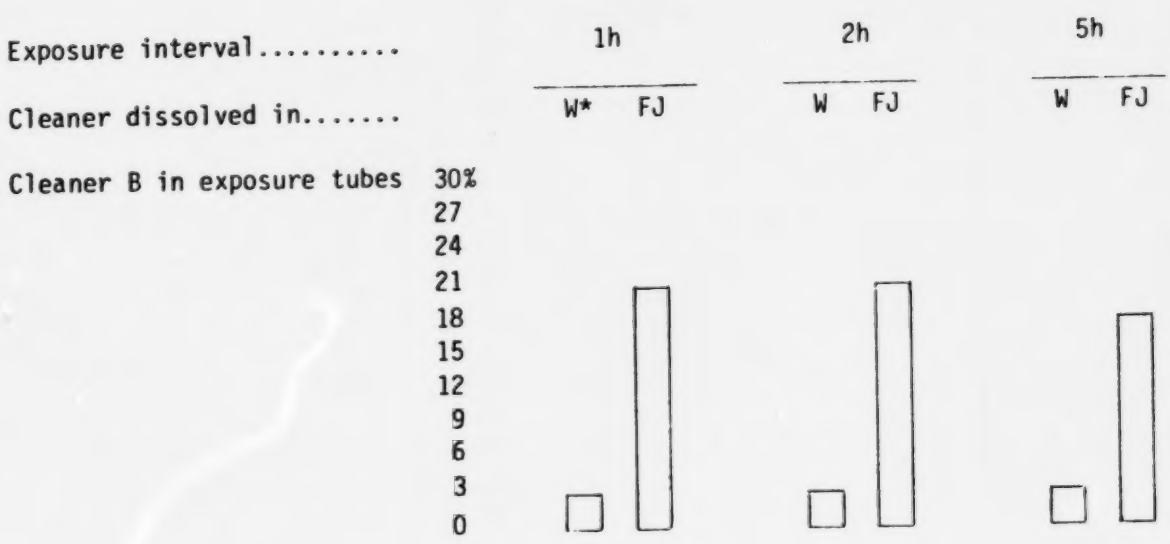
□ = growth in recovery tubes at 24h

▤ = further growth in recovery tubes at 48h

TABLE 4

ANTIBACTERIAL ACTIVITY OF CLEANER B

Acinetobacter-Moraxella 25C



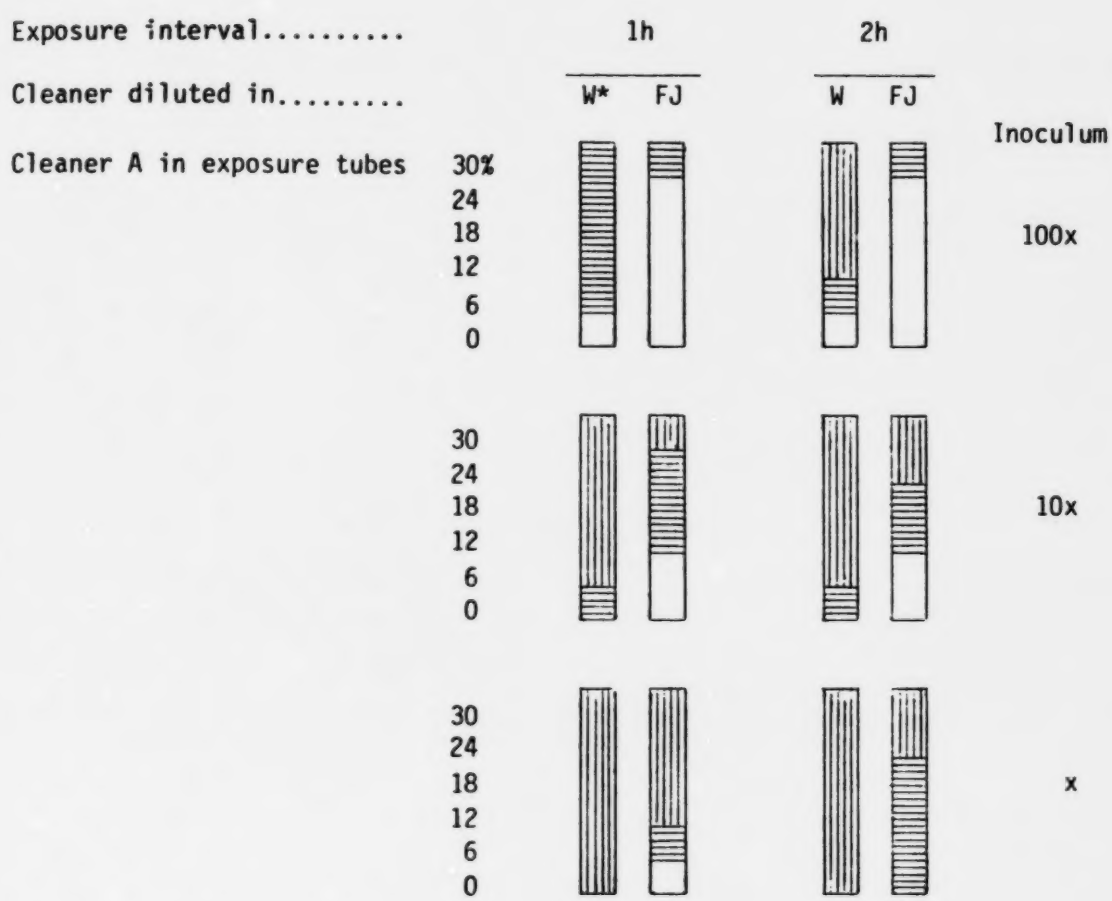
* W = cleaner dissolved in water
FJ = cleaner dissolved in fish juice

☐ = growth in recovery tubes

TABLE 5

ANTIBACTERIAL EFFECT OF CLEANER A

Pseudomonas type I 14C



* W = cleaner dissolved in water
FJ = cleaner dissolved in fish juice

□ = growth in recovery tubes, 24h at 14C

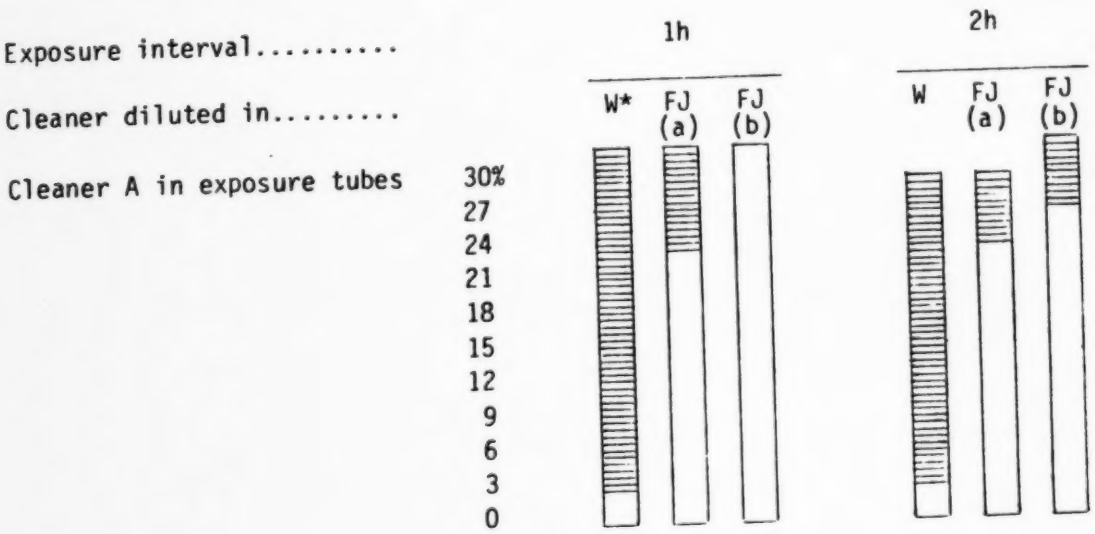
▤ = further growth in recovery tubes, 48h at 14C

▦ = " " " " & 24h at 25C

TABLE 6

ANTIBACTERIAL ACTIVITY OF CLEANER A

Pseudomonas type III/IV 25C



* W = cleaner dissolved in water
FJ(a) = cleaner dissolved in fish juice (1 part fish : 2 parts water)
FJ(b) = " " " " (1 part fish : 1 part water)

□ = growth in recovery tubes 24h

▤ = further growth in recovery tubes 48h

TABLE 7

ANTIBACTERIAL ACTIVITY OF A QUATERNARY AMMONIUM COMPOUND

Pseudomonas type I 25C

Disinfectant concentration, ppm.....		1600	1200	1000	800
Bacteria suspended in:					
saline	i*				
	ii				□
	iii				
fish juice 1:4	i				
	ii			□	□
	iii				
fish juice 1:2	i				
	ii			□	□
	iii	□	□		

* tubes i, ii, and iii in Capacity Use Dilution Method

fish juice = fish : water

□ = growth in recovery tubes

TABLE 8

ANTIBACTERIAL ACTIVITY OF HYPOCHLORITE

Pseudomonas type I 25C

Disinfectant concentration, ppm hypochlorite		1000	500	100	50
Bacteria suspended in:					
saline	i*		▤	□	□
	ii				
	iii				
fish juice	i		▤	□	□
	ii				
	iii				

□ = growth in recovery tubes at 24h

▤ = further growth in recovery tubes at 48h

* tubes i, ii, and iii in Capacity Use Dilution Method

TABLE 9 pH VALUES OF IODOPHORS DILUTED WITH SALINE OR FISH JUICE

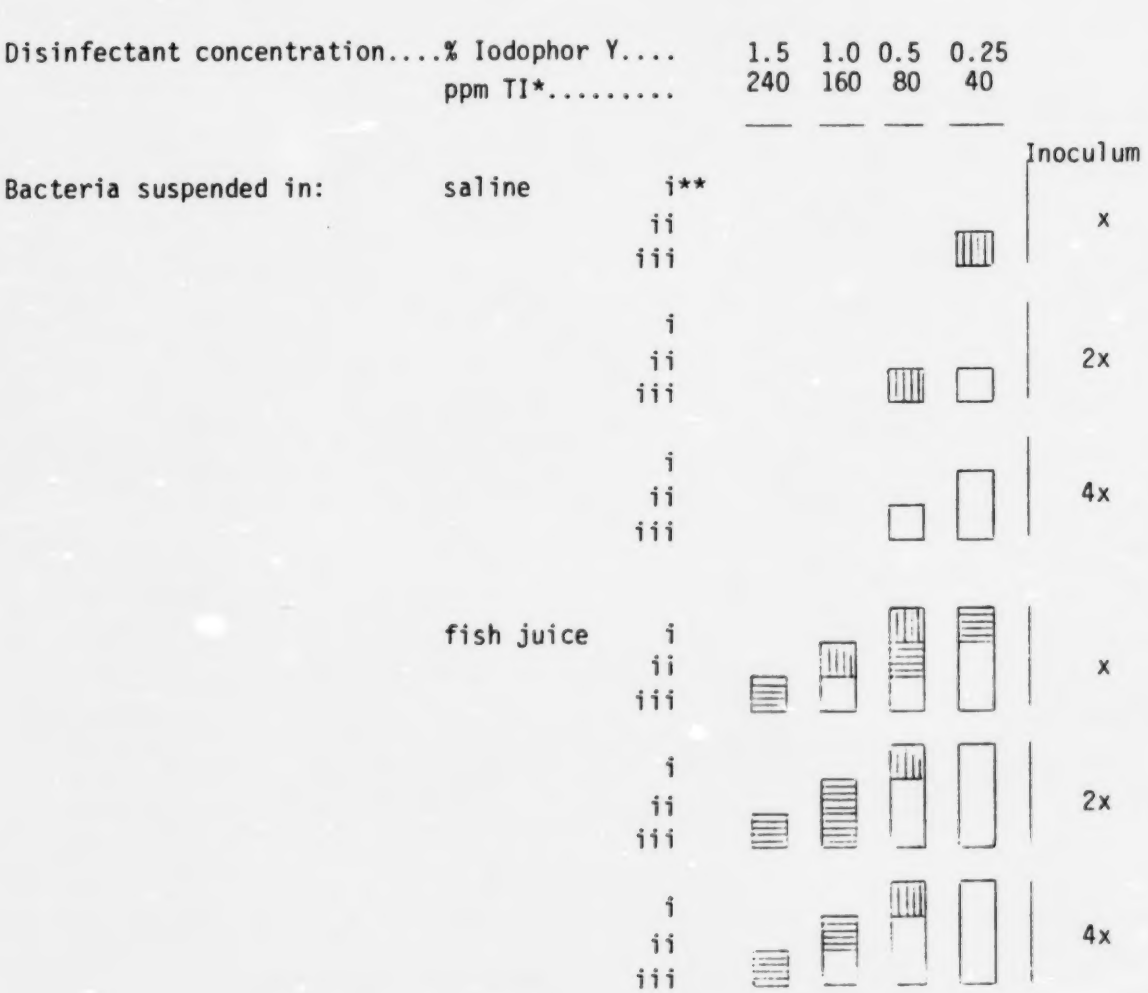
		A	B	C	D	
Concentration of iodophor, %.....		1.5	1.0	0.5	0.25	
Titratable iodine (TI), ppm.....		240	160	80	40	
Iodophor Y						
Original		3.8	4.1	4.4	4.7	
After additions						
...saline	1 ml	4.2	4.3	4.6	4.7	i*
	2 ml	4.3	4.4	4.7	4.7	ii
	3 ml	4.4	4.6	4.7	4.7	iii
...fish juice	1 ml	5.8	5.8	5.9	5.9	i
	2 ml	5.9	6.0	6.1	6.1	ii
	3 ml	6.1	6.1	6.1	6.1	iii
Iodophor Z						
Original		2.8	2.8	3.8	4.3	
After additions						
...saline	1 ml	2.8	2.9	4.0	4.4	i
	2 ml	2.9	3.0	4.1	4.4	ii
	3 ml	3.1	3.8	4.3	4.4	iii
...fish juice	1 ml	5.3	5.6	5.8	5.8	i
	2 ml	5.6	5.8	6.1	6.1	ii
	3 ml	5.9	6.0	6.1	6.2	iii

* Corresponding recovery tubes in Capacity Use Dilution Method

TABLE 10

ANTIBACTERIAL ACTIVITY OF IODOPHOR Y (no phosphoric acid)

Pseudomonas type III/IV 14C



* TI = titratable iodine
** tubes i, ii, and iii in Capacity Use Dilution Method

□ = growth in recovery tubes, 24h at 14C

▤ = further growth in recovery tubes, 48h at 14C

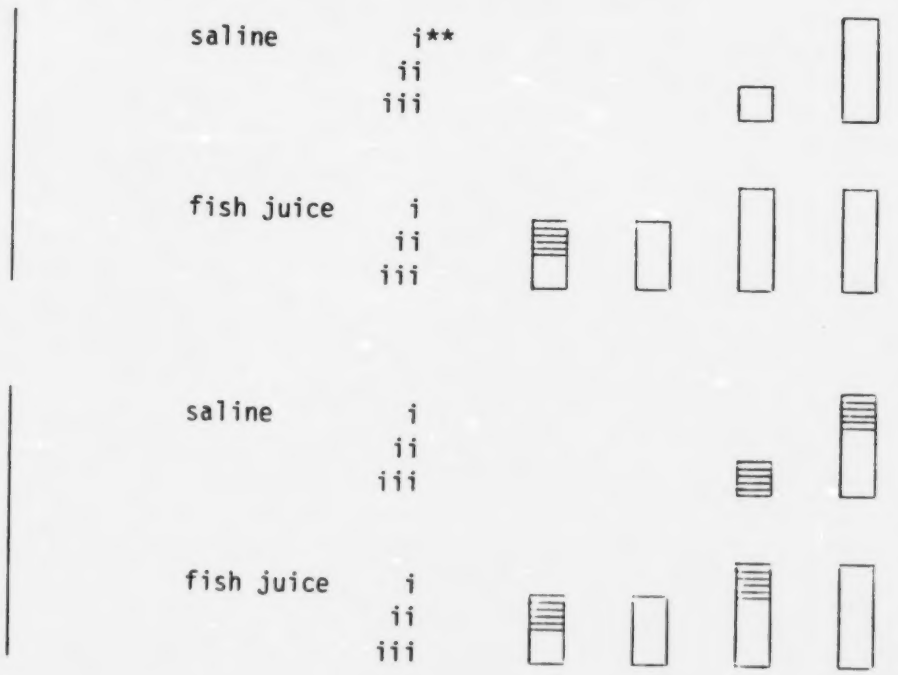
▥ = " " " " " " & 24h at 25C

TABLE 11

ANTIBACTERIAL ACTIVITIES OF IODOPHORS Y AND Z

Pseudomonas type I — 25C

Disinfectant concentration....% Iodophor....		1.5	1.0	0.5	0.25
ppm TI*.....		240	160	80	40
Bacteria suspended in:					
Iodophor Y	saline i**				
	saline ii				
	saline iii				
	fish juice i				
	fish juice ii				
	fish juice iii				
Iodophor Z	saline i				
	saline ii				
	saline iii				
	fish juice i				
	fish juice ii				
	fish juice iii				



* TI = titratable iodine

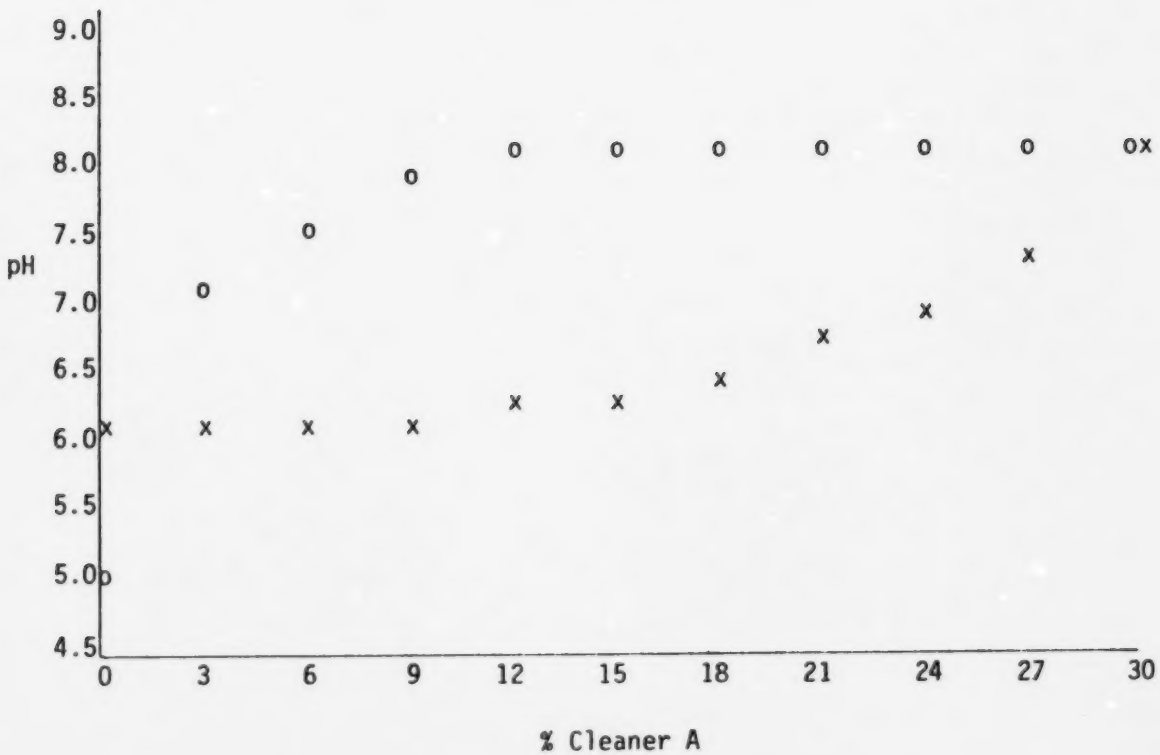
** tubes i, ii, and iii in Capacity Use Dilution Method

□ = growth in recovery tubes, 24h at 25C

▤ = further growth in recovery tubes, 48h at 25C

FIGURE 1

pH VALUES OF SOLUTIONS OF CLEANER A

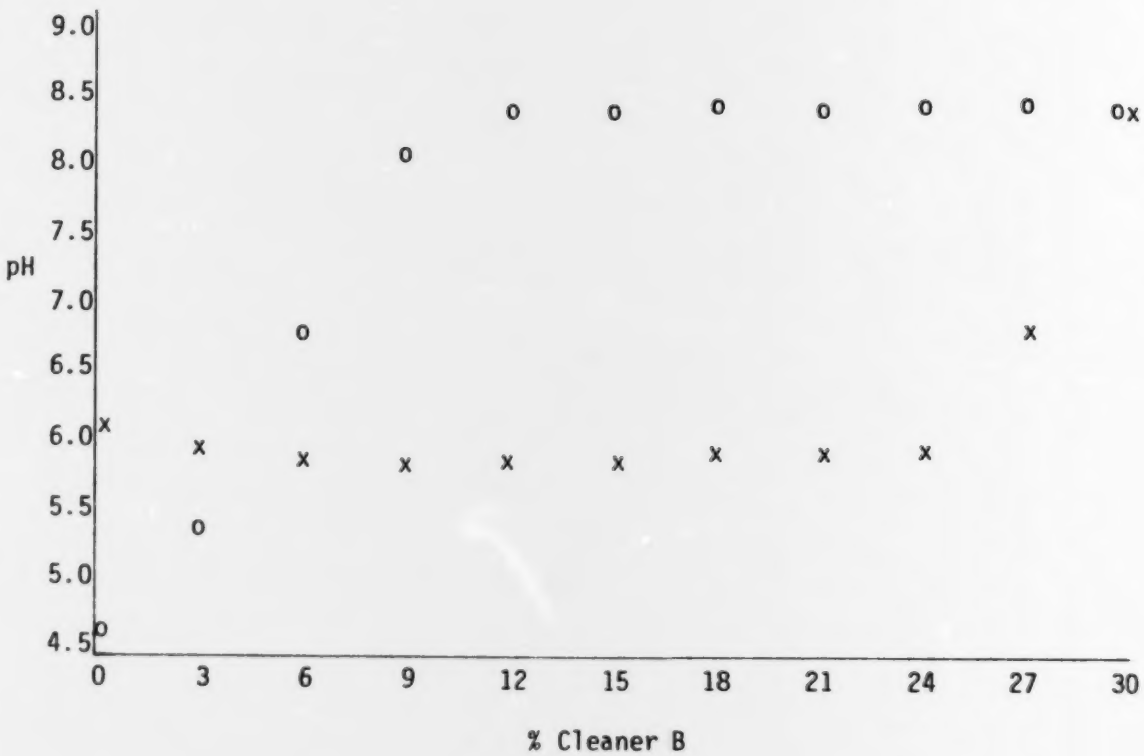


o = water solution of cleaner

x = fish juice solution of cleaner

FIGURE 2

pH VALUES OF SOLUTIONS OF CLEANER B



o = water solution of cleaner

x = fish juice solution of cleaner

FIGURE 3 Corrosion of copper coupons (25 cm^2) at 40 C as a function of time in cleaners and disinfectants. Immersion time: 12 x 1 hr intervals over a 72 hr period.